Hypothesis

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Bactofection for Cheap Liver Gene Delivery

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Bactofection for Cheap Liver Gene Delivery

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Abstract: Adeno-associated viruses (AAVs) have been used for liver gene therapy. Hemgenix and Roctavian are AAV-based treatments for hemophilia B and A, respectively. They cost $3.5 million and $2.9 million per dose, respectively. AAV vectors may eventually be cheaper to mass produce than they are currently, but a facultative intracellular bacterium-based DNA delivery system would be much cheaper for patients at this point in time, at least. Also, this approach would allow for the delivery of much larger DNA packages than AAVs can accommodate. Such a bacterial delivery system for the liver may be feasible now, and a prototype could possibly be developed rapidly.

Keywords: Bactofection; liver gene therapy; adeno-associated virus; lipid nanoparticle; Hemgenix; Roctavian

1. Introduction

Adeno-associated viruses (AAVs) can only encode ~5 kb of DNA maximum, which is not sufficient to cure certain individuals with genetic disorders wherein long stretches of nucleotides are affected [1,2]. Also, the cost of producing sufficiently high titers of AAV vectors and certain other viral vectors for therapeutic purposes is very steep [2–4].

DNA delivered by non-viral vectors, e.g., lipid nanoparticles (LNPs), has typically only been able to mediate transient expression *in vivo*. Strategies to increase the duration of transgene expression include CpG depletion, delivery vector optimization, and co-encapsulating siRNA targeting inflammatory pathways [5]. All three options reduce inflammation-induced silencing. The second may also increase endosomal escape to some extent. However, in general at least, endosomal escape is the main bottleneck for non-viral vector gene delivery [6,7]. It is possible that in most cases of LNP-mediated DNA delivery, only a few copies of DNA get to each target cell nucleus - and that they are rapidly silenced.

A synthetic bacterial vector with low immunogenicity that can deliver DNA to the liver would be of use for multiple reasons. First, it would be cheap. Second, it would be very adept at endosomal escape. Third, it could deliver large DNA constructs to use as homologous repair templates or large serine recombinase/CRISPR transposase cargo for individuals with genetic disorders wherein long stretches of nucleotides are affected [8,9]. Fourth, due to the fenestrated sinusoidal endothelium in the liver, bacteria injected intravenously would mainly end up in the liver [10].

2. Prototype for the Liver

Bactofection is a method that has been used for DNA transfer to target cells. It involves invasion of an intracellular bacterium into a target cell, endosomal rupture, and finally lysis [11]. One of the primary roadblocks to bactofection *in vivo* has been the immune response to the bacteria.

Three possible vectors would be *Escherichia coli* Nissle 1917 (EcN), *Salmonella* Typhimurium, and *Listeria monocytogenes*. Attenuated bacteria have been safely injected intravenously for cancer therapy in the past [12,13]. In fact, the bacteria may not even have to have the ability to replicate at all. Non-replicating bacteria could be generated in multiple ways [14–16]. However, if it turns out to be necessary to allow a limited amount of bacterial replication in the liver, one could administer dexamethasone to upregulate the expression of CY3PA, followed by a caged luciferin molecule that is uncaged by CY3PA [17]. The bacterial vector would express firefly luciferase and have a Deadman

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switch that is sensitive to light [18–22]. Bacteria that stray outside of the liver, where the luciferin is uncaged, would quickly stop replicating and lyse.

An *L. monocytogenes* LADD double-deleted strain could not be used here[14], however, as it cannot enter hepatocytes efficiently [23]. InlB would have to be expressed. Alternatively, a synthetic adhesin could be used to facilitate hepatocyte uptake [24,25]. Gram-negative bacteria could be myristoylation negative to decrease the immune response to the vector [26]. Additionally, the vector could display P66, a bacterial mimic of CD47, on its surface to prevent phagocytosis by Kupffer cells [27]. Dexamethasone could also be used to suppress the immune response prior to injection of the bacteria [28]. If the vector is made non-replicating through the overexpression of RelA, and possibly the suppression of DnaA and FtsZ, the immune response could also be much less severe [15,16,17].

Only two genes are necessary for EcN to invade certain human cell types and escape the endosome: the *Yersinia pseudotuberculosis* invasin and listeriolysin O [29]. The *Y. pseudotuberculosis* invasin functions as an adhesin and invasin. Another adhesin, invasin, or protein with both functions may be required for EcN to enter hepatocytes.

To increase the efficiency of bactofection, the *L. monocytogenes actA* promoter can be used to drive phage lysin production when the bacteria enter the cytosol of target cells [30]. For Gram-negative bacteria, a different strategy would be employed [31,32]. Using linear DNA could increase the efficiency of bactofection, as it may be taken up more easily through nuclear pore complexes than circular DNA [33]. A nuclear localization sequence (NLS)-containing protein could also be used to enhance nuclear gene delivery [34]. Circular DNA could be linearized by an endonuclease that is under control of the *actA* promoter. DNA ligase inhibition may be useful here. Alternatively, NLS-containing terminal proteins can be utilized that are covalently bound to both ends of a linear plasmid. This would help prevent exonuclease digestion of the plasmid DNA in the cytoplasm, as well as prevent dissociation of the NLS-containing protein [35].

Notably, it was recently shown that a linear DNA construct ~20 kb in length was able to reach the nucleus of a target cell when bound by an NLS-containing protein [35].

Moreover, it may be possible to employ a mechanism involving asymmetric division of the vector inside target cells, wherein one of the progeny cells remains viable and the other lyses to release its cargo [36–38]. The other progeny cell could continue to asymmetrically divide until at least one copy of the DNA construct reaches the host cell nucleus, at which point expression of an artificial gene product would cause it to lyse. This could also be used with a threshold effect.

3. Conclusions

Facultative intracellular bacteria could be used for liver gene delivery. They may be much cheaper than AAV-based liver gene therapies, and even LNPs. Unlike with LNPs, expression from nuclear-localized linear DNA delivered by bacteria may be durable. This approach would also allow for the delivery of large DNA packages.

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